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The influence of microbial activity on rock fluid interaction: baseline characterization of deep biosphere for Enhanced Gas Recovery in the Altmark natural gas reservoir

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Abstract

In this study the first results of the microbial monitoring within the framework of the CLEAN project are described. The microbial community of a 3.5 km deep depleted gas reservoir in Altmark, Germany was analyzed by molecular genetic techniques. Sequence analyses indicated the presence of microorganisms similar to previously identified microbes from saline, thermophilic and anaerobic environments in the deep hypersaline and hot reservoir environments. The results of the phylogenetic analyses revealed the presence of the different H₂-oxidising bacteria (*Hydrogenophaga* sp., *Acidovorax* sp., *Ralstonia* sp., *Pseudomonas* sp.), thiosulfate-oxidising bacteria (*Diaphorobacter* sp.) and biocorrosive thermophilic microorganisms.

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Microbial monitoring; deep biosphere; geological CO₂ storage

1. Introduction

The storage of CO₂ in deep geological formations is a promising technology to reduce greenhouse gas emissions into the atmosphere. Potential geological reservoirs for CO₂ (e.g.

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saline aquifers, hydrocarbon reservoirs) contain complex biogeochemical systems, including a broad diversity of minerals, brines and largely uncharacterized microbial ecosystems. Many important processes in geological systems are partly or even completely catalyzed by microorganisms. The injection of large volumes of CO_2 perturbs the environmental conditions especially pH, temperature and pressure. These changes in reservoir conditions can stimulate microbial activity, which might influence the long-term safety of CO_2 storage in the subsurface. For example, stimulation of the microbial activity by CO_2 injection might enhance the dissolution of certain minerals, thus increasing the permeability and storage capacity. Conversely, the precipitation of mineral or biological compounds may lead to reduced permeability and loss of injectivity in the reservoir [1]. Furthermore, reactions between supercritical CO_2 and rock materials might produce fluids with increased concentrations of dissolved organic compounds, which can supply the autochthonous microorganisms with energy.

The discovery of active microbial communities deep below the seafloor [2], [3], in saline aquifers [4], [5], [1] and in continental subsurface systems [6] has far-reaching implications for the planned storage of CO_2 in geological reservoirs. In these extreme environments well-adapted and highly active microorganisms exist, and play an important role in reservoir biogeochemical cycling [7].

Within the CO_2 Largescale EGR in the Altmark Natural-gas field (CLEAN) project (Fig. 1), the microbial community of the natural gas reservoir was investigated. The reservoir is nearly gas-depleted [8] and thus a suitable site for testing enhanced gas recovery processes [9]. Detailed characterisation of the reservoir and near-reservoir layers is a crucial component of the project. Mineralogy, structure and geochemistry of the reservoir rock and the cap rock as well as interaction with reservoir fluids affect the microbial community composition and its activity. This study characterized the microbial community of the deep biosphere of the Altmark reservoir by 16S rRNA gene sequence analysis.

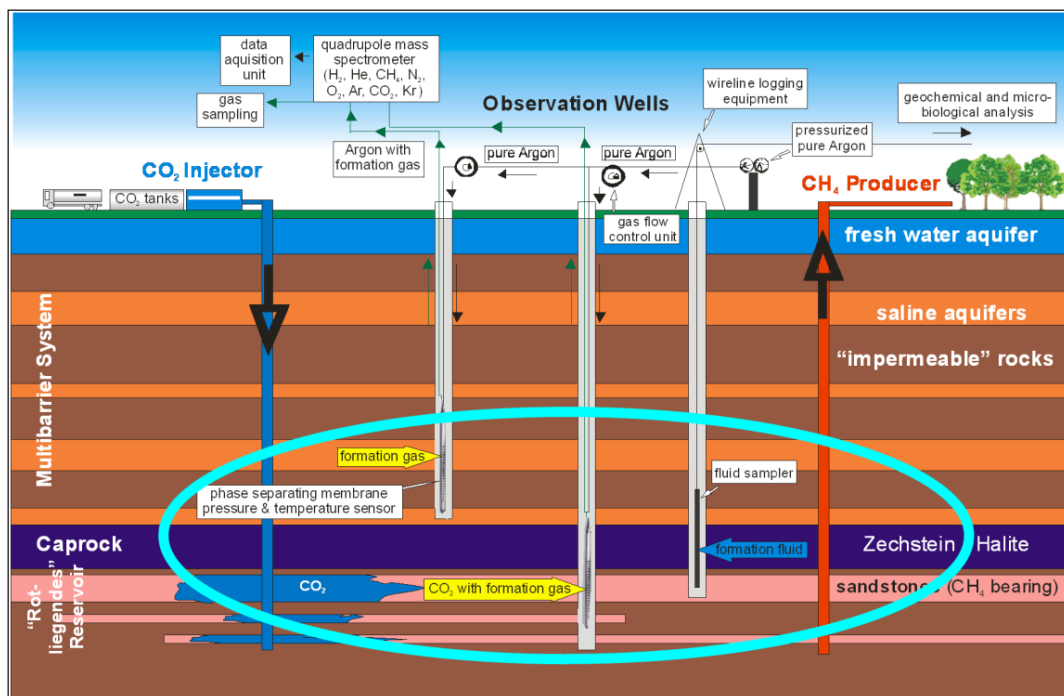


Figure 1 Schematic view of the CLEAN project monitoring system.

2. Site Description

The Rotliegend natural gas reservoir in the Altmark field is located in the German federal state Sachsen-Anhalt, south of the city Salzwedel (Fig. 2). It is estimated as the second largest onshore gas field in Europe [9]. The Scholle Altsalzwedel is composed of alternating sandstone, claystone and siltstone at a depth of approximately 3500 m [9]. It is characterized by high salinity brines (up to 420 g/l), high pressure (~ 20 MPa) and temperatures above 120 °C. Altogether, 420 wells have been drilled in Altmark since the year 1969. This study concentrates on two wells, Aaz 148 and Aaz 144, that were proposed for CO₂ injection and observation

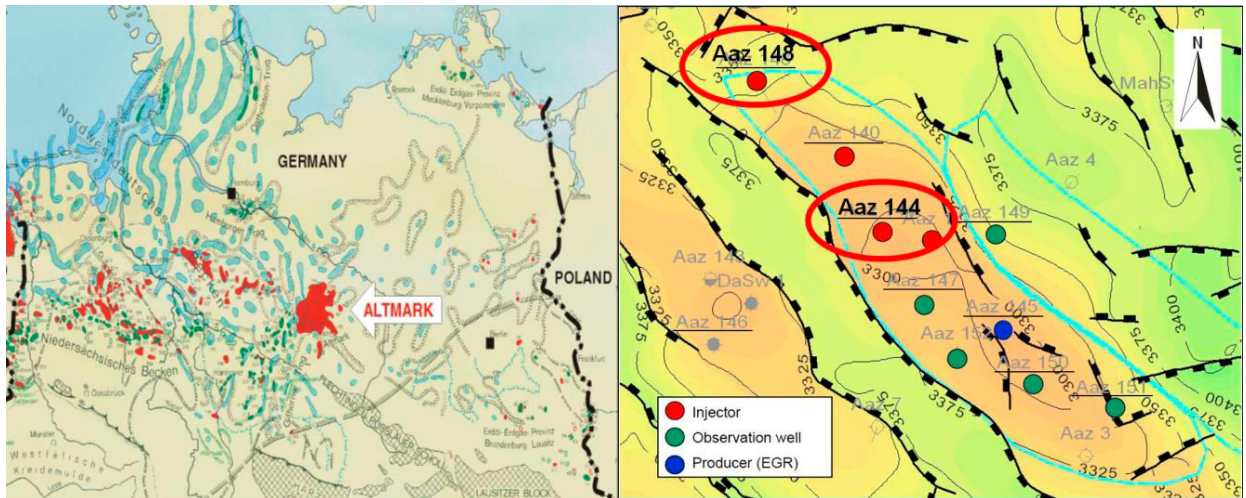


Figure 2 The gas field Altensalzwedeler Scholle in the Altmark, Germany.

3. Methods

3.1 Sampling

Fluid samples were collected from the reservoir using downhole sampling (Erdöl-Erdgas Workover GmbH, Fig. 3A) at depths of 3000 m, 3436 m and 3512 m (perforation depths) in Aaz 148 and 3420m in Aaz 144 using double ball-lining (in German DoppelKugelBüchse, DKB) and bottom hole positive displacement samplers (PDS). The DKB samplers were inserted open into the well, and lowered until the required sampling depth. The DKB was then flushed by well fluids, closed and raised to the surface. The PDS sampler is inserted into the well closed until defined depth. It then opens to take a sample, closes again and is raised to the surface. The PDS sampler was sterilized by heating at 180 °C for 2h inside the heating jacket (Leutert™), specially developed for preheating PDS sampler (Fig. 3B). The DKB sampler was cleaned in the laboratory and washed with sterilised deionised water and ethanol immediate before sampling. The pH, conductivity, temperature and other parameters were measured directly after the sampling process. The fluids were transferred aseptically into sterilised 100 to 1000 ml glass vials, refrigerated to 4 °C and immediately transferred to the laboratory for microbiological analyses. Importantly, a nitric acid treatment and water flush was performed prior to sampling in well Aaz 144 due to build up of sand deposits resulting in well clogging. About 4 l nitric acid and 4 m³ water were added, and 0.4 m³ fluids were back-produced prior to sampling in order to remove salts and rust from the well.



Figure 3 A: Sampling of reservoir fluids using depth sampling technologies. B: Heating jacket with PDS sampler inside. C: Reservoir fluids from DKB and PDS sampler.

3.2 Geochemical analyses

Electrical conductivity, pH and fluid temperature were measured at the surface using a portable pH/mV/temperature meter (WTW). The total organic carbon content was determined using a TOC-analyser (Dimatec GmbH) according to DIN EN 1484-H3.

3.3 Molecular approaches applied to the reservoir fluids

Genetic profiling of amplified 16S rRNA genes were applied for characterization of the microbial community by PCR–Single-Strand-Conformation Polymorphism (PCR-SSCP) and Denaturing Gradient Gel Electrophoresis (DGGE). For DNA extraction, microbial cells were concentrated by filtration of the reservoir fluids on 0.2 μm filter units (Millipore).

Nucleic acids were extracted from preserved filters with Ultra Clean DNA Isolation Kit (Mo Bio Laboratories) according to manufacturer's suggested protocols with few modifications. 16S rRNA subunits were amplified by polymerase chain reaction (PCR) and nested PCR using different *Bacteria* and *Archaea*-specific primers. PCR products were analysed by electrophoresis via SSCP [10] and DGGE methods [11]. The obtained 16S rRNA gene sequences were compared with the sequences available in the GenBank database using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

4. Characterization of the microbial community

The physiological environment of the Altmark reservoir (Table 1) represents an extreme environment for microbial life [12], [13], [14].

Table 1 Geochemical analysis of the downhole fluid samples for the well Aaz 148. (Geochemical analyses for the fluids from the well Aaz 144 are not presented here due to the possible influence of the well treatment prior sampling on geochemical data.)

Well	Depth (m)	pH	Salinity (g/l)	DOC (mg/l)
Aaz 148	3000	5.7	395	123.1
Aaz 148	3436	5.9	370	70.2
Aaz 148	3512	5.5	420	299.4

High temperature is postulated to be the one of the most limiting factors controlling microbial growth and survival at great depths [3], [15], [16]. However, first results of the Altmark baseline survey indicated the presence of microorganisms in this deep hot subsurface environment. The sequence analyses revealed the presence of several H_2 -oxidising bacteria (*Hydrogenophaga sp.*, *Adicdovorax sp.*, *Ralstonia sp.*, *Pseudomonas sp.*) in all fluid samples, thiosulfate-oxidising bacteria (*Diaphorobacter sp.*) in the fluids from the well Aaz 148 at 3000 m and 3512 m depths, and biocorrosive thermophilic microorganisms, which have not previously been cultivated, in the fluids from Aaz 144 (3420 m) and Aaz 148 (3512 m) wells (Fig. 4, Tab. 2, 3). Furthermore, several uncultivated microorganisms were found, that were similar to representatives from other saline, hot, anoxic, deep environments [17], [18], [19], [20]. Given possible heterogeneity of the reservoir fluid resulting in microhabitats with lower temperature, our results suggest that it is possible that we were able to identify the autochthonous reservoir community. *Ralstonia*, *Prevotella* and *Pseudomonas* species were found in deep sediments [18], [21]. Together with *Hydrogenophaga* those species were described as “Knallgas” bacteria, which are characterized by chemolithotrophic growth [17]. Among those Knallgas bacteria thermophilic species are known [22]. Biocorrosive thermophilic communities were also described [20]. Although well Aaz 144 was treated with acid and water prior to sampling, no possible contaminants were identified yet. Furthermore, recent studies showed that upper temperature limit for the microbial life was underestimated [23], [24]. For example, Kashefi and Lovely [25] were able to cultivate archaea at 121°C, that were isolated from a 300 °C environment. Other archaeon from deep hydrothermal vent, *Methanopyrus kandleri*, was shown to be able to survive and reproduce at 122 °C [26]. Furthermore, heterotrophic microorganisms were found in 140 °C hot environments [27]. Therefore, our results suggest, that fingerprinting methods were sensitive enough to detect the inhabitants of the reservoir. However, high amplification rates due to low cell counts increase the possibility of the contamination, and this possibility can not be entirely excluded.

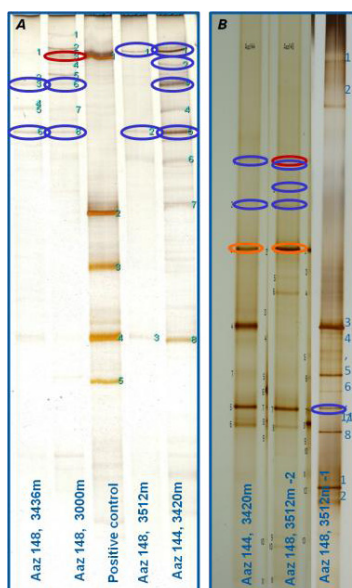


Figure 4 The PCR-SSCP analyses (A) and DGGE analyses (B) of the downhole fluid samples from the Rotliegend reservoir. Selected bands are marked with circles: red for thiosulphate-oxidising bacteria, violet for H_2 -oxidising bacteria, orange for biocorrosive microorganisms that have not previously been cultivated.

Table 2 Affiliation of the PCR-SSCP fragments (Fig. 4A)

SSCP Fragments	Similarity [%]	Organism	References
144-3420-1 148-3512-1	100	<i>Acidovorax sp.</i>	Indiragandhi, P. et al. Cultivable bacterial strains isolated from two-spotted spider mite <i>Tetranychus urticae</i> population resistance to pyridaben, unpublished
148-3000-2	99	<i>Comamonas nitrivorans</i>	Etchebehere, C. et al. <i>Comamonas nitrivorans</i> sp. nov., a novel denitrifier isolated from a denitrifying reactor treating landfill leachate
148-3000-3 148-3512-1 144-3420-1	99	<i>Diaphorobacter nitroreducens</i>	Jroundi, F.F. et al. Culturable carbonatogenic bacteria activated by a consolidating treatment of deteriorated monumental calcarenite stone, unpublished Sun, J. et al. Isolation and characterization of 4-chlorophenol degrading strain from cooking water, unpublished
148-3000-5	96	<i>Prevotella sp.</i>	Adolphe, Y. et al. Minimal diversity in grease trap revealed by molecular analysis of bacterial community, unpublished Ueki, A. et al. <i>Prevotella paludivivens</i> sp. nov., a novel strictly anaerobic, Gram-negative, hemicellulose-decomposing bacterium isolated from plant residue and rice roots in irrigated rice-field soil, 2007
148-3000-6 148-3436-3 144-3420-2 144-3420-3	99	<i>Pseudomonas sp.</i>	Wei, Y. et al. Phylogenetic analysis the diversity of <i>Pseudomonas</i> strains isolated from Mingyong glacier, unpublished
148-3000-8 148-3436-6 148-3512-2 144-3420-5	94	<i>Ralstonia sp.</i>	Konstantinidis, K.T. et al. Microbial diversity and resistance to copper in metal-contaminated lake sediment, 2003
144-3420-8	100	Unidentified bacterium	Boivin-Jahns, V. et al. Bacterial diversity in a deep subsurface clay environment, 1996

Table 3 Affiliation of the PCR-DGGE fragments (Fig. 4B)

DGGE Fragments	Similarity [%]	Organism	References
144-3420-8	99	Uncultured beta Proteobacterium	Sahl, J.W. et al. Novel microbial diversity retrieved by an autonomous rover in the world's deepest phreatic sinkhole, 2009
148-3512-1	95	<i>Diaphorobacter sp.</i>	Pham, V.H. et al. <i>Diaphorobacter oryzae</i> sp. nov., isolated from a thiosulfate-oxidizing enrichment culture, 2009
148-3514-1 144-3420-1	95	<i>Acidovorax sp.</i>	Kellermann, C. et al. CO ₂ fixation potential and occurrence of autotrophic microorganisms in an aquifer situated in an agriculturally used area, unpublished
148-3512-2 148-3512-4 144-3420-3	96	Uncultured bacterium clone	Duncan, K.E. et al. Biocorrosive Thermophilic Microbial Communities in Alaskan North Slope Oil Facilities, 2009
148-3512-3 148-3512-6	96	<i>Ralstonia sp.</i>	Lin, S. et al. Chlorophyll-containing bacterium from deep-sea water, unpublished
148-3512-10	98	Uncultured bacteria	Lin, L.H. et al. Planktonic microbial communities associated with fracture-derived groundwater in a deep gold mine of South Africa, 2006
148-3512-11	98	<i>Hydrogenophaga sp.</i>	Gangwar, P. et al. Bacterial diversity of soil samples from the western Himalayas, India, 2009
148-3512-11	99	Uncultured Actinobacterium	Wang, J.J. et al. Diversity of free-living bacteria along with salinity gradient, 2009

Identification of the biocorrosive thermophilic microorganisms could be of great importance for the technical progress of the long-term CO₂ storage technique. Recent investigations showed that members of this group were able to rapidly and massively change the permeability of the injectivity in the area most proximal to the well bore [28], [29]. Thermophilic sulphate reducing bacteria and other thermophilic microorganisms (methanogenic archaea, Fe-reducing bacteria etc) were discovered to contribute to corrosion of metallic oilfield pipelines [20].

The results reported here are the first to be described for the hot deep fluid in Altmark reservoir. The occurrence of biocorrosive microbial communities could be important for the long term safety of the storage operation. Further analyzes in order to characterize life in hot saline reservoir fluids using specific primers, molecular cloning and activity measurements are planned.

5. Acknowledgements

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